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Selective Cellular Expression of Tissue Factor in Human Tissues

Implications for Disorders of Hemostasis and Thrombosis

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Tissue factor (TF), the primary cellular initiator of the coagulation protease cascade, is implicated in having important roles in hemostasis, thrombogenesis, inflammation, and the cellular immune response, although the cytologic distribution of TF in tissues has yet to be described. This study used epitope-defined monoclonal antibodies to human tissue factor for immunohistochemical localization of TF in normal human tissues. TF was selectively expressed in tissues and was associated with cells rather than extracellular matrix. It was anatomically sequestered from blood, being undetectable in endothelium and peripheral blood cells. TF was present in vascular adventitia, organ capsules, epidermis, and mucosal epithelium. Most dermal and submucosal fibroblasts were negative. Except for alveolar macrophages and possibly dendritic cells of some lymphoid follicles, tissue macrophages did not express TF; (expression was demonstrable in LPS stimulated monocytes). Cerebral cortex, renal glomeruli, and cardiac myocytes were additional sites of prominent TF expression. Based on the cellular distribution of TF, it is hypothesized that intravascular initiation of coagulation requires induced expression by intravascular cells, and that the normal distribution of TF represents a hemostatic "envelope" ready to activate coagulation when vascular integrity is disrupted. (Am J Pathol 1989, 134:1087–1097)

Tissue factor (TF), the primary cellular initiator of the coagulation protease cascade, is a cell membrane-associated protein that serves as the receptor and essential cofactor for factors VII and VIIa.[1] The bimolecular complex of TF and factor VII or VIIa activates factors IX and X by limited proteolysis, leading ultimately to thrombin generation and fibrin formation. As a potent initiator of coagulation, TF is believed to have a critical function in hemostasis and thrombogenesis.[2–4] Cytokines, immune complexes, and other physiologic and pathologic mediators can induce TF expression in monocytes or endothelial cells, suggesting an integral involvement of TF in the pathologic processes of inflammation and thrombogenesis.[4–13] In addition, TF is implicated in the effector phase of the cellular immune response and in the pathogenesis of certain infections.[14–20]

Knowledge of the localization of TF in tissues, particularly within the vasculature, is necessary to understand the pathobiology of the thrombotic, hemorrhagic, and a variety of diseases where local activation of coagulation is indicated by the presence of fibrin. Ubiquitous tissue distribution of TF has been suggested by the constitutive production of TF by fibroblasts, smooth muscle cells, and other selected cells in culture, and the recovery of TF from various organs.[1,21–23] Contact between TF and plasma coagulation factors does not appear likely, however, because the coagulation system is normally not activated to a significant degree. Among normal cell types, lack of expression of TF activity has been found in all circulating blood cells and endothelial cells in culture.[22–25] Of these, only monocytes and endothelium can be induced to express TF by various mediators. Demonstration of these latter phenomena has altered the prevailing view of thrombogenesis from one of exposure of preformed TF in vascular cells with injury to one in which vascular cells play an active role by synthesizing and expressing TF and modulating other procoagulant properties in response to specific stimuli.[4] The delineation of the *in situ* expression (or lack thereof) of TF within normal vessels and extravas-

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cular tissues that is necessary to provide a morphologic foundation for any proposed mechanism of thrombogenesis and hemostasis has not been established, however.

Until recently, lack of precise delineation of human TF or reagents for its cellular localization had hampered efforts to elucidate the role of tissue expression of TF in hemostasis and in thrombotic and inflammatory diseases. Recently, TF has been purified from human brain, the cDNA cloned, and monoclonal antibodies derived to the purified protein.[26–31] These studies have shown that human TF is a novel protein, lacking significant homology with other known proteins. Three monoclonal antibodies that react with at least two distinct epitopes on TF, two reacting with a functional region of TF and one to a nonfunctional region, were used for immunohistochemical studies of TF distribution in normal human tissues. Based on the cellular distribution of TF, it is hypothesized that intravascular initiation of the coagulation protease cascade in thrombogenesis, disseminated intravascular coagulation, and related pathology requires induced expression by intravascular cells, and that TF represents a hemostatic extravascular "envelope" to which exposure of plasma proteins on disruption of vascular integrity leads to activation of coagulation. This hypothesis and the pattern of TF distribution also can account for the pattern of bleeding observed in hemophilia.

Materials and Methods

Monoclonal Antibodies

Three murine monoclonal antibodies (MAb) reactive with human TF (TF9-9C3, TF9-9B4, and TF9-10H10) were selected from a panel of MAbs derived from immunizations with purified human brain TF, the isolation and characterization of which are reported elsewhere.[27] Specificity for TF has been confirmed by dot blot and Western blot reactivity. Two of the antibodies recognize functional epitopes on TF, inhibiting TF initiated coagulation of human plasma by more than 90% and inhibiting specific binding of factor VII to cultured cells expressing TF by more than 80%. The third antibody recognizes a distinct nonfunctional epitope, and was used to ensure that TF would still be recognized in tissues that may have bound factor VII *in vivo*.[32] All three antibodies bind strongly to cultured human cell lines expressing TF, while reactivity with cells not expressing TF is absent.

For immunohistochemical studies, the antibodies used were from hybridoma culture supernatants, diluted to working concentrations of approximately 0.1 μg/ml in reagent diluent (2% bovine serum albumin, 0.05% thimerisol in 0.05 M TRIS-HCl, 0.9% NaCl, pH 7.4). An irrelevant IgG1K mouse MAb (ATCC TIB-115)[33] was used as a control, at equivalent concentrations in reagent diluent, also from hybridoma culture supernatant. Monoclonal antibody GCA7, specific for smooth muscle actin,[34] was purchased from Enzo Biochem (New York, NY).

Tissue Procurement and Preparation

Unfixed tissues were obtained from nonpathologic portions of surgical or autopsy specimens from the UCLA Medical Center. Samples were obtained within 6 hours of surgical removal (usually within 1 to 2 hours), during which time specimens were kept at 4 C, or in the case of autopsy material, within 24 hours of death. Three-millimeters thick sections were embedded in O.C.T. compound in 15 × 15 mm cryomolds, and rapidly frozen by immersion in an isopentane-dry ice bath, then stored at −70 C. The age of patients from whom tissue was used ranged from 17 to 65 years, except for thymic tissues, which were from children under 10 years.

Immunohistochemistry

Immunohistochemistry was performed on freshly prepared cryostat sections (4 to 6 μ thickness) using a modified ABC technique.[35,36] Sections were fixed in 2% paraformaldehyde in phosphate-buffered saline (PBS, 0.01 M sodium phosphate, 0.14 M NaCl, pH 7.4) for 2 to 5 minutes, immersed in 0.1 M glycine in 0.005 M TRIS-HCl, 0.9% NaCl (TBS wash) for 10 minutes, rinsed in distilled water for 30 seconds, and air dried for 20 to 30 minutes. Before primary antibody incubation, sections were incubated sequentially in avidin (10 U/ml) and d-biotin (1 mg/ml) for 20 minutes each (each followed by three 2-minutes washes in TBS) to block endogenous avidin and biotin binding activities,[37] and in blocking solution (5% dry milk solids, 1% horse serum, 0.1% sodium azide in 0.01 M TRIS-HCl, 0.14 M NaCl, pH 7.4) for 20 minutes to reduce nonspecific binding. Primary antibody was incubated on sections in humidified chambers for 60 minutes at 37 C, followed sequentially by biotinylated horse antiserum IgG, H+L (Vector Laboratories, Burlingame, CA, 1:400 in reagent diluent) for 30 minutes at 37 C and streptavidin-horseradish peroxidase complex (DETEK 1-hrp, Enzo Biochemical, 1:100 in reagent diluent) 30 minutes at 37 C, with each step followed by 3 TBS washes over 10 minutes. Bound peroxidase was detected using the chromogenic substrate 3-amino-9-ethyl carbazole (0.02% in a freshly prepared solution of 0.02 M sodium acetate buffer, pH 5.1, and hydrogen peroxide, 0.03%) incubated for 10 minutes at 37 C. Sections were then washed in distilled water, counterstained with Mayer's hematoxylin, and mounted in glycerol-gelatin. Some tissues were also ex-

amined using a commercial kit (ABC Elite, Vector Laboratories, Burlingame, CA) according to the manufacturers instructions. Sequential sections of each tissue were stained with the control MAb in parallel with specific antibody to assess nonspecific staining, which in most instances was negligible.

Peripheral blood leukocytes were prepared from buffy coat layers of freshly drawn heparinized blood. After hypotonic lysis of erythrocytes,[38] cells were resuspended in Medium 199 plus 10% fetal bovine serum at approximately 5×10^5 cells/ml. Cytocentrifuge preparations on poly-L-lysine-coated slides were made directly or after incubation of cell suspensions with 1 μg/ml LPS (*Escherichia coli* 0111:B4, List Biologicals, Campbell, CA) at 37 C for 4 hours in 5% CO_2. Slides were immediately fixed in 2% paraformaldehyde plus 0.2% glutaraldehyde in PBS for 60 minutes at 4 C, permeabilized by immersion in 100% methanol at −20 C for 60 seconds, then stained as described above except for the following: slides were not air dried; 5% nonfat dry milk was included in all TBS washes; endogenous peroxidase was blocked by incubation of slides with 0.1 M periodic acid for 5 minutes then 0.02% sodium borohydride for 2 minutes, performed after the secondary antibody step.[39]

TF Quantitation in Muscle Tissues

Skeletal and cardiac muscle were collected as described above and frozen at −70 C before use, then ground to a fine powder in a morter and pestle in liquid nitrogen. The homogenate was diluted in 4 parts by weight of 0.05 M TRIS, 0.9% NaCl, pH 7.4 (TRIS-saline buffer) and filtered through a 100 μ steel mesh. Total protein concentrations of the filtrates were determined using a dye binding assay (Bio-Rad Laboratories, Richmond, CA.[40] Procoagulant assays were performed on samples diluted to 10 μg of protein per ml in TRIS-saline buffer, using a plasma recalcification time technique.[22] TF was quantitated by reference to a standard curve prepared using purified human brain TF reconstituted in phospholipid vessicles. These assays were performed in duplicate, with addition of 10 μg/ml of either an inhibitory monoclonal antibody to human TF or control monoclonal to the muscle homogenates prior to assay.

Results

Specificity and General Characteristics of Staining

Evaluation of selected tissues (spleen, brain, skin, kidney, placenta) with each MAb alone and with the three in combination yielded identical results. This colocalization of MAbs directed against at least two distinct epitopes, including one involved in functional association with Factor VII, gave confidence that none of the three MAbs were reacting with unsuspected crossreactive epitopes on other molecules, or that the epitopes were obscured by association with Factor VII, the ligand for TF. Supportive evidence that the molecule detected immunohistochemically was functional TF was obtained by quantitating TF procoagulant activity in skeletal and cardiac muscle. Cardiac muscle, which was positive by immunohistochemistry, had 17.5 times more procoagulant activity than skeletal muscle, which was negative immunohistochemically (Table 1). Performance of the assay in the presence of a monoclonal antibody inhibiting TF function, showed that greater than 90% of the procoagulant activity measured was due to TF.

Table 1. *TF Procoagulant Activity in Skeletal and Cardiac Muscle*

	ng TF/mg protein*	Fraction inhibited by anti-TF MAb
Cardiac muscle (N = 3)	119 ± 65	93.9%
Skeletal muscle (N = 3)	7 ± 4	91.5%

In preliminary studies, the potential effect of tissue storage at 4 C for varying periods of time before freezing was examined. Portions of skin, brain, and placenta received within 60 minutes of removal from the body were frozen immediately and after 8 and 24 hours while being kept moist at 4 C. Immunohistochemistry performed at one time on sections from each showed no evidence of either loss or new appearance of antigen over time. Also, no differences were noted in the patterns of reactivity or relative intensity between like tissues from surgical or autopsy sources. These observations indicated that the TF epitopes detected were relatively stable in tissues within the range of conditions encountered in this study.

Immunohistochemical localization of TF showed differential expression among the various tissues examined (Table 2, Figures 1–17). No substantial differences were noted between tissues from different individuals. A general finding was that TF appeared to be cell associated, within the resolution of light microscopy. At no site was there evidence for extracellular matrix localization. Chromogen localization in positive cells was usually diffuse rather than focal or limited to the cell outline.

Blood Vessels

TF in blood vessels was predominately localized to cells of the adventitia, being undetectable in endothelium and

Table 2. *Summary of TF Expression in Human Tissues*

Skin	Epidermis	+++
	Dermis	−
Gut	Mucosa	+++
	Submucosa	−
	Muscularis	V (+)
Vessels	Intima	−
	Media	V (+)
	Adventitia	++
	Capillaries	−
Heart	Myocardium	+++
	Endocardium	−
	Cardiac valves	−
Lung	Bronchial mucosa	++
	Bronchial submucosa	−
	Alveolar septae	+
	Alveolar epithelial cells	++
	Alveolar macrophages	V (++)
Brain	Meninges	+
	Cerebral cortex	+++
Kidney	Glomeruli	+++
	Tubules	−
	Interstitium	−
Spleen	Capsule	++
	Trabeculae	+++
	Splenic cords	−
	Lymphoid areas	−
Liver	Hepatocytes	+
	Kupffer cells	−
	Biliary duct epithelium	−
Adrenal glands	Cortex	−
	Medulla	+
Peripheral nerve	Schwann cells	++
	Axons	−
Skeletal muscle	Myocytes	−
	Perimysium	−

−, absent; +, weak intensity; ++, moderate intensity; +++, strong intensity; V, variably present, with intensity, when present, in parentheses.

variably present in cells composing the media (Figures 6,7,9,18,19). Aorta and elastic arteries had prominent adventitial localization that was distinctly cell associated and not present in connective tissue matrix. The medial layer of these vessels showed variable weak to moderate reactivity in some areas. Intima was consistently negative. Most muscular arteries and larger arterioles also had distinct adventitial reactivity, although in some locations (kidney and gut submucosa) this was absent or minimal but in other locations (spleen, superficial cerebral vessels) was exceptionally intense. (These differences were consistent among specimens from different sources and could not be attributed to individual variation or to sample handling.) Cells in the media of these vessels and in smaller arterioles was generally negative, although they were occasionally weakly to moderately positive, in focal areas. Smaller arterioles also had adventitial cell localization but this was often not prominent and, as with larger vessels, was absent in kidney and gut.

Capillaries and postcapillary venules did not react at any site. Adventitial cells of small to medium sized veins generally reacted similarly to those of arteries of comparable size and location. In large veins, TF was expressed in cells that were interspersed between bundles of smooth muscle cells. Because a defined medial layer is thin to nonexistent in these vessels,[41] TF expressing cells were irregularly present near the intima.

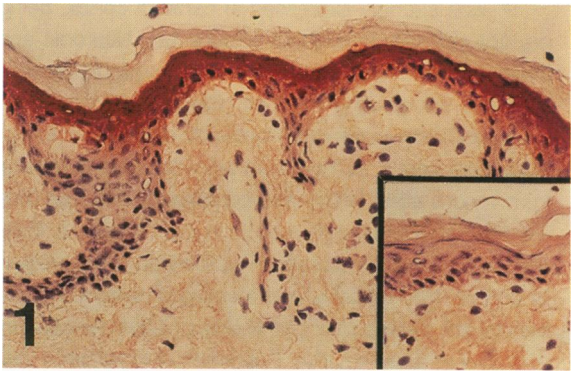
Parallel sections of larger arteries and veins were reacted with MAb to TF and to smooth muscle actin to determine whether the TF positive cells in adventitia were actually of smooth muscle origin. In both arteries and veins, adventitial cells expressing TF did not contain immunoreactive smooth muscle actin, suggesting they were fibroblasts (Figures 18, 19).

An exception to the absent or irregular reactivity of vascular smooth muscle was observed in umbilical cord and placental arteries and veins, where medial smooth muscle cells expressed TF uniformly with moderate intensity. Endothelium was negative, whereas adventitial cells reacted intensely, as did the stromal cells in the umbilical cord matrix.

Peripheral Blood Cells

Unstimulated peripheral blood leukocytes were uniformly negative (Figure 8a). Incubation of cells with endotoxin for 4 hours induced immunohistochemically detectable TF in monocytes but not neutrophils or lymphocytes (Figure 8b). Eosinophils, basophils, erythrocytes, and platelets also were negative. Lymphocytes in spleen, thymus, and lymph nodes were negative. Macrophages in tissues and derived cells lining vascular channels in spleen and liver

Figure 1. *Skin (inset: control MAb): epidermis is strongly positive while dermis is negative (×231).* **Figure 2.** *Colonic mucosa (inset; control MAb): epithelium is positive while lamina propria is negative except for cells of the subepithelial fibroblastic sheath (×231).* **Figure 3.** *Vaginal mucosa: association of TF expression with maturation of the squamous epithelium is marked (×92).* **Figure 4.** *Bronchial mucosa (small bronchus): epithelium is positive, staining more strongly in basally located cells; adjacent submucosa is negative (×369).* **Figure 5.** *Urinary bladder mucosa: intense staining occurs in upper submucosa wherease transitional epithelium is only faintly positive (×231).* **Figure 6.** *Large meningeal artery (L, lumen): advential cells are strongly positive while media and intima and negative (×231).* **Figure 7a.** *Carotid artery (L, lumen): adventitia is positive, media weakly positive, and intima negative (×92).* **b:** *Iliac vein (L, lumen): scattered cells in the adventitia are positive, the intima is negative (×231).* **Figure 8a.** *Peripheral blood leukocytes (unstimulated): neutrophils, lymphocytes and monocytes (M) are all negative (×577).* **b:** *Peripheral blood leukocytes exposed to LPS for 4 hours: a monocyte (M) has been induced to express TF whereas neutrophils and lymphocytes remain negative (×577).* **Figure 9a.** *Arteriole, uterus: adventitia of vessel is positive; vascular intima and media and uterine smooth muscle is negative (×231).* **b:** *Arteriole and small vein, muscular wall of colon: no staining of adventitia is seen, in contrast with vessel in panel a; autonomic ganglion (arrow) is positive, smooth muscle of muscularis very weakly positive (×231).*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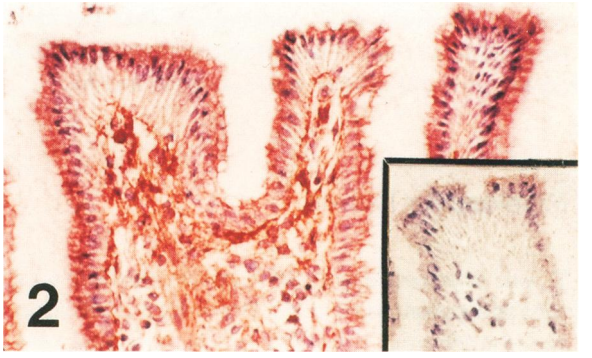
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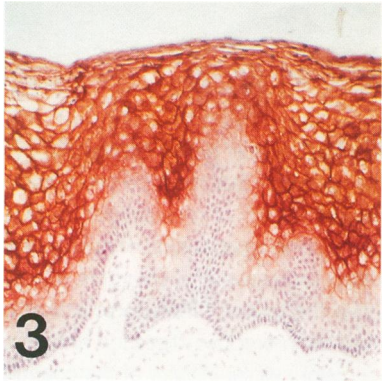
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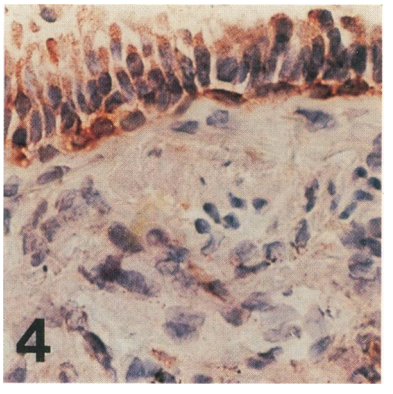
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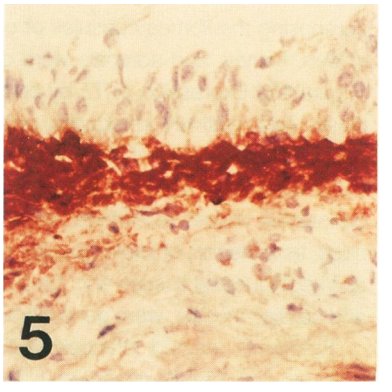
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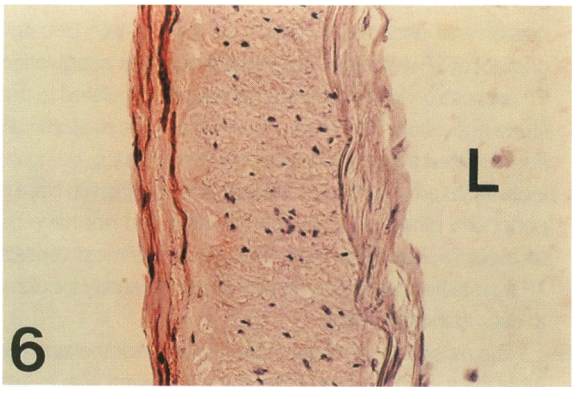
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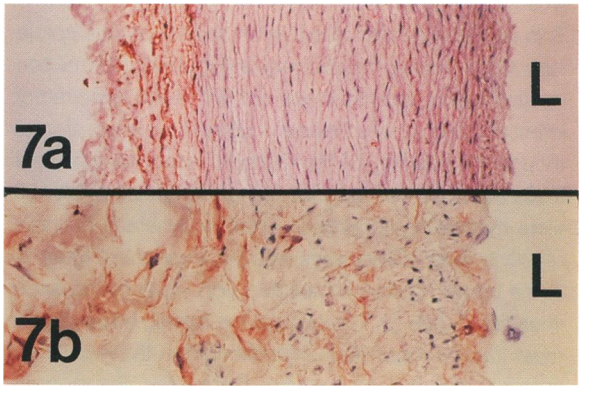
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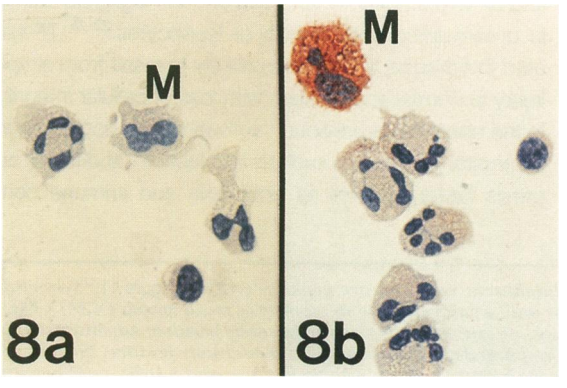
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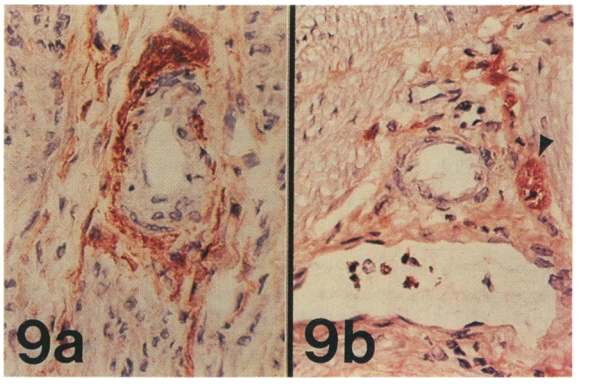
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also were negative. Exceptions were alveolar macrophages, which were variably positive with weak to moderate intensity, and cells of dendritic morphology in germinal centers of reactive follicles in spleen, tonsil, and gut.

Extravascular Tissues

TF was selectively expressed in various organs and tissues as summarized in Table 2. Prominent expression occurred in epidermis, bowel and respiratory mucosa, cerebral cortex, myocardium, and renal glomeruli. Also, cells composing the fibrous capsules of organs such as liver, spleen, kidney, and adrenals were consistently positive (Figure 13). Expression of TF by connective tissue fibroblasts was relatively infrequent, occurring most prominently in the adventitia of blood vessels, and in the subepithelial fibroblastic sheath of bowel mucosa. Fibroblasts in connective tissues elsewhere, whether densely collagenous, as in dermis or joint capsules (Figure 11), or loose, as in submucosal tissues and the perimysium of skeletal and cardiac muscle, were generally negative.

Epidermis reacted intensely, with the strongest expression consistently observed in the granular cell layer, whereas the basal layer was weak to negative (Figure 1). This pattern also was observed in the squamous epithelium of the oropharynx and vagina (Figure 3). Dermal connective tissue and capillaries did not react. Respiratory, intestinal, and urinary bladder mucosae were positive, although the intensity of the reaction varied. Mucosal epithelium and derived submucosal glands of larger bronchi were moderately to strongly positive, whereas epithelial reactivity in smaller airways was more variable (Figure 4). In the bowel, epithelium reacted with weak to moderate intensity (Figure 2). Throughout the bowel, however, the most intense localization was present in the 1 to 2 cell thick layer of the subepithelial fibroblastic sheath (pericryptal fibroblasts) located immediately subjacent to the epithelium. Other cells in the lamina propria did not express TF. In the urinary bladder, transitional epithelium was weakly positive and in some samples, negative (Figure 5). Intense reactivity occurred in a band of otherwise nondescript upper submucosal stromal cells, whereas cells deeper in the submucosa were negative.

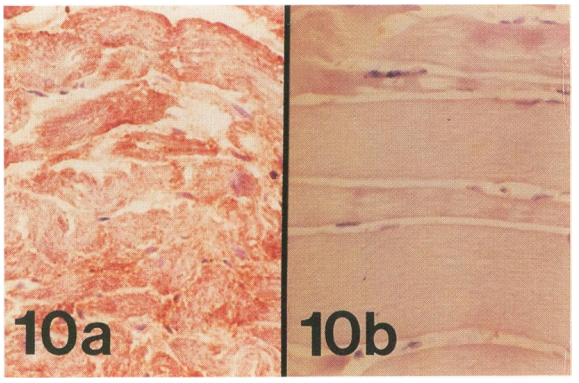
TF was strongly expressed in cerebral cortex, being present diffusely throughout (Figure 12). Neuronal and other cell bodies were positive, but not more prominantly than the surrounding neuropil. Cardiac myocytes expressed TF with a uniform cytoplasmic distribution (Figure 10a). This was in striking contrast with skeletal muscle from various sites, which was consistently negative (Figure 10b), as was smooth muscle in most locations. In renal parenchymal structures, TF was limited to the glomeruli (Figure 15). Epithelial and mesangial cells of the glomerular tuft reacted strongly, whereas glomerular capillary endothelium was negative. Epithelium of Bowman's capsule also was positive.

Discussion

Maintenance of a potent, rapidly active hemostatic system is a physiologic necessity that must be structured in a manner that minimizes the risk of intravascular coagulation. This is achieved by limiting activation of coagulation, inhibiting the activated proteases and cofactors that are formed, and by active fibrinolysis.[42] Pathologic activation of coagulation is key to thrombotic diseases and in inflammation. TF is a major cellular initiator of the coagulation protease cascade, functioning as the cell surface receptor and essential cofactor for Factor VII.[1] Because contact of TF with blood is sufficient to initiate coagulation, TF expression in vessels must be strictly regulated to preclude activation. This study shows that TF is anatomically sequestered from the blood in the normal state, being selectively expressed at sites isolated from plasma. Normal peripheral blood cells and endothelium did not have detectable TF, nor did smooth muscle cells of most vessels. TF associated with vessels was predominantely localized to cells in the adventitia.

The absence of detectable TF in endothelium and other cells in contact with blood is consistent with *in vitro* and *ex vivo* studies, which have shown negligible activity in unstimulated endothelium or leukocytes.[22-25] TF-initiated thrombosis is therefore unlikely to result from simple injury to normal endothelium with loss of cellular integrity, in the absence of previous "activation." Endogenous inflammatory mediators such as interleukin-1 and tumor necrosis factor, as well as endotoxin and immune com-

Figure 10a. *Cardiac muscle: myocytes are positive (×231).* **b:** *Skeletal muscle: myocytes are negative (×231).* **Figure 11.** *Synovium, knee: synovial cells and connective tissues of stroma are negative or only weakly positive in vicinity of small vessels (×231).* **Figure 12.** *Cerebral cortex: cortical parenchyma is strongly positive in diffuse pattern; meninges are moderately positive; adventitial staining is present around a small meningeal vessel (×92).* **Figure 13a.** *Lymph node, cortex: capsule is moderately positive, lymphocytes are negative (×369).* **b:** *Liver: capsule is moderately positive, whereas hepatocytes are weakly positive and Kupffer cells negative (×369).* **Figure 14.** *Lung: alveolar macrophages and epithelial cells are positive (×369).* **Figure 15.** *Glomerulus, kidney: glomerular interstitium and cells of Bowman's capsule are positive; endothelium of capillary loops appear negative, as do surrounding renal tubules (×231).* **Figure 16.** *Placental villi: trophoblast is negative whereas internal stroma is positive, strongest around vessels (×231).* **Figure 17.** *Spleen with arteriole and adjacent lymphoid follicle: adventitia is strongly positive as are scattered cells in the center of the follicle (×92).*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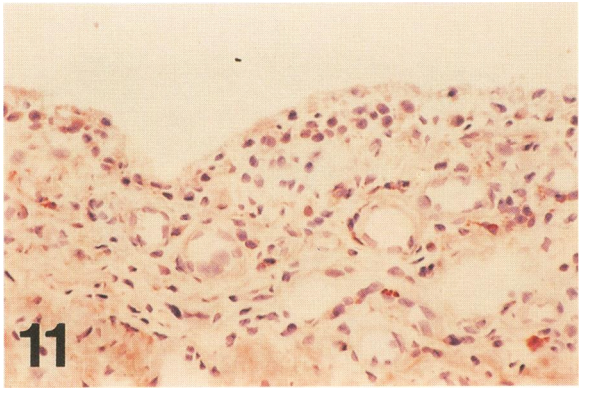
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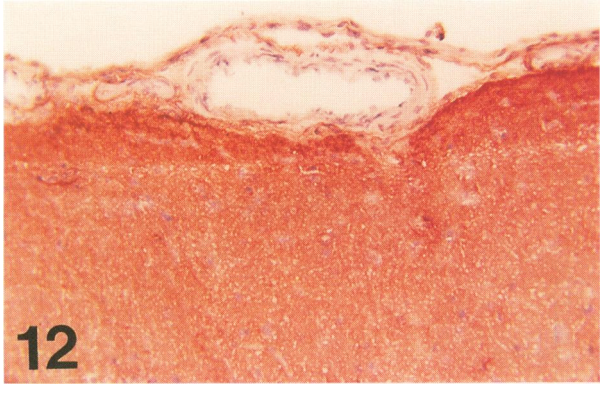
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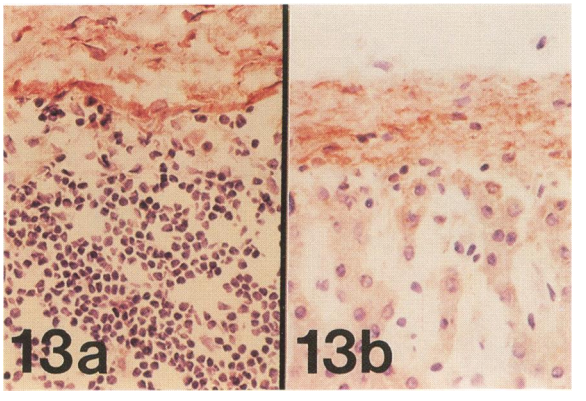
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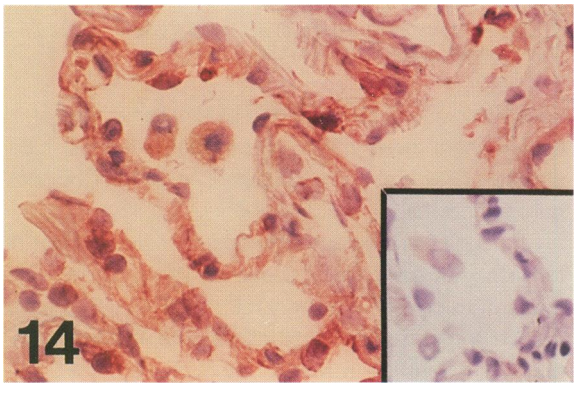
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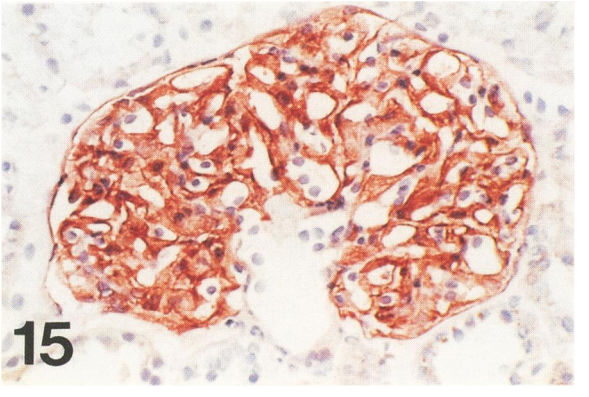
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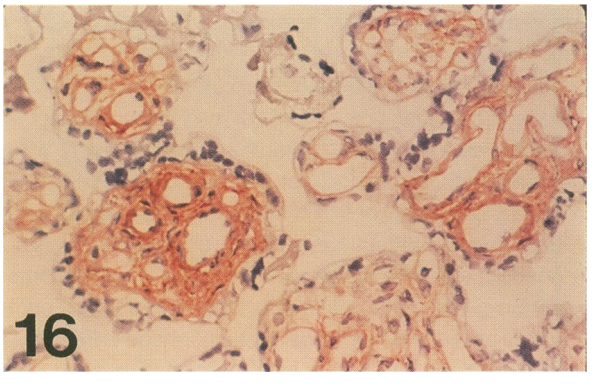
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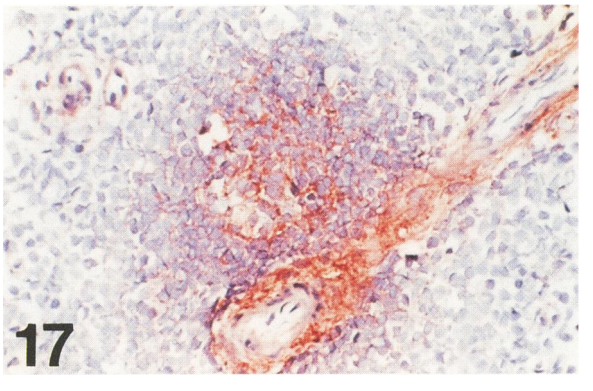
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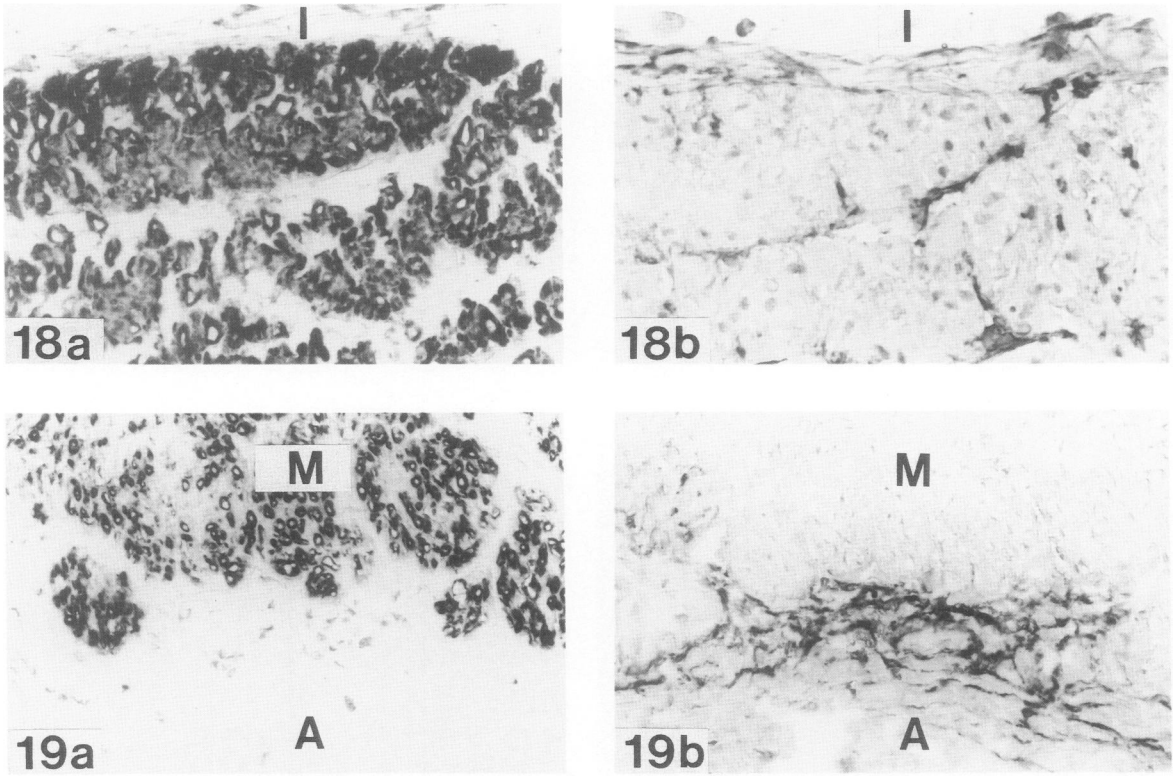

Figure 18. *Saphenous vein: parallel sections incubated with anti-smooth muscle actin MAb* (a) *and anti-TF MAb* (b) *showing lack of smooth muscle actin expression in TF positive cells which surround bundles of smooth muscle (I, intima; ×369).* Figure 19. *Aorta, junction of media and adentitia: parallel sections incubated with anti-smooth muscle actin MAb* (a) *and anti-TF MAb,* (b) *showing lack of smooth muscle actin expression in TF positive cells in adventitia (M, media; A, adventitia; ×231).*

plexes, can induce endothelial cell TF expression and are implicated in the thrombogenic process, although endothelial cell expression of TF *in vivo* remains to be demonstrated.[6–10] Monocytes also are capable of being induced to express TF by various stimuli and also are implicated in thrombogenesis as well as extravascular fibrin deposition in inflammation.[5,11,13,14] Monocytes and macrophages at sites in contact with blood such as liver and spleen did not express detectable TF, nor did these cells at many extravascular sites, lung being a prominent exception.

We were able to demonstrate immunohistochemically, as has been indicated by other techniques,[24,25] that the monocyte, when stimulated with endotoxin, an etiologic agent in septic shock, is the only normal peripheral blood cell to express tissue factor. This is consistent with previous implications of this cell as a potentially responsible initiating cell in thrombogenesis and in cellular immune responses.

The observed distribution of TF is consistent with its presumed primary function in hemostasis. TF was generally localized in what may be thought of as an "envelope" distribution: it surrounded blood vessels in adventitia, encased organs in fibrous capsules, and was present in epidermis and mucosal epithelium and subjacent stromal cells in skin and internal mucosae, thus providing a continuous hemostatic barrier with the external environment. In brain, where even minor hemorrhage may be catastrophic, TF was strongly expressed throughout the cortical parenchyma.

Although there are as yet no documented cases of TF deficiency (a complete absence of which may be fatal in utero) to confirm a primary role for TF in hemostasis, collective information viewed in light of its anatomic distribution supports such a role. Although Factor VII deficiency has been known to be associated with impaired hemostasis, the defects associated with Factors VIII and IX deficiencies have implicated the contact activation pathway as being of major importance. Recent studies have shown that TF-VIIa complex can activate Factor IX as well as Factor X, and that the former pathway is responsible for the majority of TF initiated Xa formation at low TF concentrations.[43,44] Studies by Broze et al[45] suggest that the recently described lipoprotein-associated coagulation inhibitor is responsible for the latter phenomenon. It is particularly interesting that the sites most commonly affected by spontaneous hemorrhage in Factor VIII or IX deficient patients, deep skeletal muscle and joints, had undetectable to low TF expression. Clinical studies in hemophilic patients who are unresponsive to replacement therapy because of inhibitory antibodies have shown that

infusions of Factor VIIa can stop bleeding.[46,47] Such responses with enhancement of TF-dependent activation of coagulation suggest an important hemostatic role for the presumed low levels of TF present at those sites. Also, our observations of high levels of TF expression in epidermis are consistent with a recent study that showed activation of coagulation to be the earliest hemostatic event in bleeding time wounds.[3] Such activation occurred in healthy individuals as well as in Factor VIII and IX deficient people, but was impaired in those with Factor VII deficiency, implicating TF rather than the contact activation pathway as the initiating mechanism. In this setting, the high level of TF expression in epidermis would be expected to preferentially promote direct X activation, making the response independent of IX activation, as was actually observed. Early thrombin generation may be an important mechanism of platelet activation in the primary hemostatic response in such wounds, which has until now been considered to be largely independent of the coagulation protease cascade.

Although much work remains to establish the relative importance of the TF and contact activation pathways in hemostasis, this study is fully consistent with and further supports previous observations that TF has a major role in this essential function. TF did not appear to be present in extracellular matrix, the putative site of contact activation, but rather was cell associated, thus providing the body with complementary rather than duplicated sites for coagulation activation.

The foregoing discussion assumes that the TF detected immunohistochemically in this study was functionally active. Two of the three MAbs used reacted with functional epitopes on TF. Also, tissues known to be rich in TF, such as brain and placenta, reacted strongly and we were able to demonstrate substantially more procoagulant activity, attributable to TF, in an immunohistochemically positive tissue, cardiac muscle, than in a comparable tissue that was negative, skeletal muscle. One study reported finding very little procoagulant activity in isolated renal glomeruli of normal kidneys, however, a site we consistently found to be strongly positive.[48] It is possible that the TF in glomeruli is predominately extracellular, and lacking in function because it would not be associated with membrane lipid required for activity. Further work will be necessary to reconcile these observations, and to determine whether TF at other sites of localization is functionally active.

Of importance also is the unknown sensitivity of immunohistochemistry in detecting low levels of TF in cells. This is complicated by a lack of knowledge concerning the levels of TF expression that may be physiologically significant. Studies by Dvorak and colleagues with an animal model have demonstrated extravascular fibrin deposition in dermis occurring after induction of increased vascular permeability.[49] In conjunction with knowledge that cultured fibroblasts express tissue factor, it was postulated that TF is normally expressed by dermal fibroblasts *in vivo*. Our inability to detect TF in human dermis could reflect some limitations of sensitivity of the analyses, among other explanations that include species differences in local TF expression. Interestingly, cultured human fibroblasts express substantial levels of TF which are detectable immunohistochemically (data not shown).

The nonuniform distribution of TF throughout the body shows that TF expression is significantly regulated in various cell types. The findings in two tissues in particular were indicative of an important association of TF expression with cellular differentiation. Among muscular tissues, TF was strongly expressed in cardiac muscle, variably expressed in smooth muscle, and was undetectable in skeletal muscle. In epidermis, TF expression was absent to weak in the germinative basal layer and strongest in the more differentiated outer layers. This potential link between squamous differentiation and TF expression also was suggested by the presence of TF in Hassell's corpuscles in the absence of expression in other thymic epithelium. An important extension of the finding of differential expression of TF in normal tissues will be evaluation of its expression in their neoplastic counterparts, and the association of thrombosis and malignancy.

TF expression by cells not normally positive, either through induction by biologic mediators or loss of regulation, may be important in various biologic and pathologic processes. As discussed above, this is probably of particular importance in thrombosis and inflammation in which endothelium and monocytes can be induced to express TF. The variable expression of TF by smooth muscle and fibroblasts at different locations suggests that these cells also may modulate TF expression in response to biologic stimuli. Although much of the distribution of TF in normal tissues can be understood in terms of its procoagulant function, its selective expression at sites not of obvious importance for hemostasis raises the question as to whether it may have additional biologic functions.

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